



Cytheris Announces Publication of IL-7 Primate Study Showing Rapid and Massive T cell Homing to the Gut

Results suggest potential of IL-7 in stimulating the T-cell repopulation of the gut, which is inaccessible to HAART therapy and constitutes a potent viral reservoir where HIV-1 continues to replicate and suppress immune function

Paris (France) – May 12, 2009 – Cytheris SA, a clinical stage biopharmaceutical company focused on research and development of new therapies for immune modulation, today announced publication of data from a study in a non-human primate model identifying a new critical function of Interleukin-7 (IL-7) that induces massive and rapid T-cell migration from the blood into various organs, including lymph nodes, parts of the intestine and the skin. The study points towards the importance of evaluating the potential of IL-7 in combination with highly active antiretroviral therapy (HAART) in stimulating the T-cell repopulation of the gut, known to be a latent HIV reservoir where the virus can continue to replicate and suppress immune function. The massive T-cell depletion of the GI tract early in the course of HIV infection opens the patient to the effects of opportunistic infections and malignancies which are frequently associated with a weakened immune system in this patient population.

The paper entitled “Injection of Glycosylated Recombinant Simian IL-7 Provokes Rapid and Massive T-cell Homing in Rhesus Macaques” is prepublished online in *Blood*, the Journal of the American Society of Hematology (Beq S et al, April 7, 2009; [doi: 10.1182/blood-2008-11-191288](https://doi.org/10.1182/blood-2008-11-191288)).

Studies such as the one described in this paper deal with the fundamental role of the gut in harbouring the HIV virus,” said Michel Morre, DVM, President and CEO of Cytheris. “This is the question that lies at the heart of the challenge for improving the efficacy of antiretroviral therapy in GI lymphoid tissue. It may also be key to developing vaccines that provide more than a peripheral blood response by addressing the critical issue of mucosal immunity.”

About the Study

In this study, conducted at the Institut Pasteur, Paris, and designed to measure early T-cell homing in the first hours post-injection, five healthy Rhesus macaques were subcutaneously inoculated with 80µg/Kg of body weight of recombinant glycosylated simian IL-7 (R-sIL-7gly).

As observed in IL-7-treated human patients, all R-sIL-7gly-treated animals demonstrated a strong peripheral lymphopenia during the first day following injection. Notably, despite the fact that T-cell increase was not observed at Day 7 in one of the macaques (designated a poor responder), the initial decrease in lymphocyte counts was also observed in this animal. In contrast, two non-injected

control animals sampled on the same schedule as the treated monkeys did not show a significant change in their circulating lymphocyte counts.

Four months later, when all the measured parameters had returned to baseline levels, a second injection of R-sIL-7gly given to two of the previously treated monkeys led to a similar drop in circulating lymphocytes.

Prior to euthanizing the animals, the investigators observed a strong and rapid T-cell migration out of the blood, with 70% of the circulating T-cells disappearing, including recent thymic emigrants, naïve, CM and EM T-cells in both CD4+ and CD8+ populations. At the same time, these T-cells up-regulated several chemokine receptors implicated in homing mechanisms, while plasma concentration of a number of chemokines/cytokines specifically implicated in migratory phenomenon was significantly increased.

In order to confirm that R-sIL-7gly injection effectively triggers T-cell homing to the lymph nodes, gut and skin, tissue samples from two animals euthanized 24 hours after R-sIL-7gly injection, one animal euthanized at Day 7 and from a fourth non-injected animal were subjected to immunohistological labeling with anti-CD3 monoclonal antibodies.

In these tissue samples, T-cell infiltration was observed in the skin and the *lamina propria* of the ileum, the colon and the rectum. Quantifying CD3+ T-cells in 7 to 10 fields (0.09 mm² each) randomly selected from four slides for each organ confirmed that the number of CD3+ T-cells per field was significantly increased by Day 1 in the skin (p=0.001), the ileum (p=0.003), the colon (p=0.018) and the rectum (p=0.05). In all organs but the colon, T-cell numbers remained significantly higher at Day 7 as compared to the control animal. In contrast, the density of CD3+ T-cells was not significantly modified in the lymph nodes.

These data confirm that R-sIL-7gly induces T-cell homing into various non-lymphoid organs including the *lamina propria* of several parts of the gut (ileum, colon and rectum) and skin. Moreover, the expression of CCR7 and CXCR4 on circulating T-cells, the increase of CXCL12 plasma concentration and the production of CCL19 and/or CCL21 mRNA in lymph nodes also suggest homing into secondary lymphoid organs.

Similarly, the production of CCL19 in the ileum and the rectum and that of CCL21 in the jejunum suggest that R-sIL-7gly injection also triggers T-cell migration into the lymphoid follicles of the gut, where massive T-cell proliferation subsequently occurs.

Human and Non-Human Primate Studies

The IL-7 induced T-cell proliferation indicated in the long-term follow-up to the Phase I/II study discussed in a recently published paper ("Enhanced T cell recovery in HIV-1-infected adults through IL-7 treatment" published online in *The Journal of Clinical Investigation*, Lévy, Y et al, 2009, Vol. 119, No. 4) is now broadly confirmed by studies in non-human primates such as the one described here and others conducted by Cytheris in collaboration with the Seattle Biomedical Research Institute (SBRI), the Vaccine and Gene Therapy Institute (VGTI) and the Oregon National Primate Research Center (ONPRC) at Oregon Health and Science University, Emory University.

The focus of these non-human primate studies is the gastrointestinal tract, where the extraordinary size of the GI mucosa facilitates what is recognized as a fundamental role in the pathogenesis of HIV-1 infection. It is well known that the HIV virus is able to survive in these mucosal tissues of the human GI tract that effectively act as a viral reservoir. Current HAART therapy, which has been successful in reducing viral loads and increasing T-cells in the blood, has been largely ineffective in attacking the virus in the mucous membrane of the GI tract.

Left hiding in the gut lymphoid tissue, the HIV virus continues to replicate and suppress immune function by depleting its preferred target, the memory CD4⁺ T-cells which constitute the body's defence against the invading virus and whose decline precedes the profound reduction in CD4⁺ T cells circulating in the blood. This then leaves the body potentially vulnerable to a variety of opportunistic infections and/or progression to full-blown AIDS.

Summary and Potential Implications

The findings based on animal models and human studies emphasize the potential stability of the IL-7 effect on the production of T-cells over time as well as its possible impact in stimulating T-cell proliferation in the lymphoid tissue layer in the mucous membrane of the GI tract. By stimulating the body's immune response through T-cell migration into the gut mucosa where the viral reservoir hides, and thus taking the therapeutic battle directly into a known conduit for HIV entry, early infection and viral dissemination, IL-7 may eventually be shown to play an important therapeutic role in subverting HIV disease pathogenesis.

The sustained immunological efficacy seen in the long-term follow-up of the previously published Phase I/II trial (Lévy, Y et al, 2009. *The Journal of Clinical Investigation*, Vol. 119, No. 4) suggests that IL-7 may provide an important avenue for reconstituting the immune system and inducing broad spectrum proliferative activity of CD4⁺ and CD8⁺ T-cells in the blood, lymph nodes and small intestine, a key therapeutic effect in achieving long term disease stability in HIV-infected patients.

About Interleukin-7

Investigational recombinant human Interleukin-7 (r-hIL-7) is a critical growth factor for immune T-cell recovery and enhancement. Cytokines that signal via the common gamma chain (γ_c) represent promising therapeutics based upon their potential to augment T cell expansion and increase the effectiveness of immune based therapies. Within this family, IL-7 is a prototypic homeostatic cytokine, produced constitutively by non-lymphoid cells. Its receptor (IL-7R α) is expressed on resting T cells, then rapidly down-regulated following T cell activation or IL-7 signaling.

IL-7 is essential for T cell development in mice and humans and for T cell homeostasis since it is required to maintain naïve CD4⁺ and CD8⁺ T cells *in vivo*. IL-7 levels rise in serum and tissues following T cell depletion and fall upon recovery.

In preclinical studies, IL-7 therapy exerts marked effects on T cell immune reconstitution in mice and primates. IL-7 augments effector and memory responses to vaccination in mice with preferential enhancement of responses to weak

subdominant antigens. In preclinical models, IL-7 therapy augments anti-tumor responses leading to improved survival when combined with anti-tumor vaccines.

Clinical trials conducted on more than 110 patients in Europe, North America and Taiwan suggest the potential of IL-7 in expanding and protecting CD4+ and CD8+ T-cells. Cytheris is currently conducting multiple clinical studies of IL-7 in HIV, HCV and cancer.

About Cytheris – www.cytheris.com

Cytheris SA is a privately held clinical-stage biopharmaceutical company focused on research and development of new therapies for immune modulation. These drugs aim at reconstituting and enhancing the immune system of patients suffering from cancer, chronic viral or bacterial infections such as HCV, HBV and HIV, or lympho-depleting treatments such as chemotherapy, radiotherapy, bone marrow transplantation (BMT) and hematopoietic cell transplantation (HCT). The company operates from its headquarters and laboratories in Issy-les-Moulineaux, a suburb of Paris, and its U.S. subsidiary in Rockville, Maryland.

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